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AMERICAN
JOURNAL OF BOTANY

VOL. IV OCTOBER, 1917 NO. 8

TAXONOMIC CHARACTERS OF THE GENERA ALTERNARIA AND MACROSPORIUM

JOHN A. ELLIOTT

I. INTRODUCTION

Alternaria and Macrosporium are among the most universally distributed and most common forms of the Fungi Imperfecti, embracing, according to the "Sylloge Fungorum" of Saccardo, 41 species and varieties of Alternaria and 189 species and varieties of Macrosporium, these numbers including some synonyms but not the new species which have been described since the publication of the last volume of Saccardo's work. Some species, such as *Alternaria solani* (E. & M.) J. & G., *A brassicae* var. *nigrescens* Peglion, and *Macrosporium sarcinaeforme* Cav. are well known and destructive parasites, but the great majority are saprophytes or have been described from non-important hosts. The ascigerous stages of a few species are known, the connection in all such cases being with the genus Pleospora.

Even a casual survey of the literature dealing with the genera in question would reveal the fact that the generic names, Alternaria and Macrosporium, are in many cases used synonymously in dealing with the best known of the parasitic species. This condition could be due either to there being no basis for distinction between the two genera, or to this basis being ill defined. The studies of the writer were undertaken with the hope of adding to the knowledge of these two genera. The work was necessarily limited and the result is in no way of the nature of a monograph.

II. HISTORICAL

The genus Alternaria was described and figured by Nees (15), *A. tenuis* being the type and only species described. The description

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is incomplete and in some particulars inaccurate, but it is definite and complete enough to leave little doubt that what Nees described was what is now generally recognized as Alternaria.

Fries (7) described the genus Macrosporium, differentiating it from Cladosporium, Helminthosporium, and Sporodesmium. The muriform spore, now given as one of the characters of the genus, is not mentioned in the generic description, otherwise it fits the present current conception fairly well. Having dropped the genus Alternaria, Fries makes no mention of it in his description of Macrosporium.

Macrosporium and Alternaria are placed by reason of their muriform spores in the section Dictyosporae of the family Dimidiaceae of the order Moniliales, the muriform spores separating them from the genera Cladosporium and Helminthosporium, which in some species are in many particulars similar. Among the Dictyosporae there is little basis, as the genera are described, for separating Stemphylium, Septosporium, or Mystrosporium from Macrosporium. The separation of the genera Alternaria and Macrosporium rests solely on the catenulation of spores in the former genus. The fact that many of the species of Alternaria now recognized were first described as Macrosporiums indicates the uncertainty of this basis for generic distinction. In the specific descriptions in both genera, while mycelium, conidiophores, and spores may all be taken into consideration, spore characters are the most used basis for distinction.

The question of the validity of the separation of the two genera arose over the study of their ascigerous connection with *Pleospora herbarum* Tul. The Tulasne brothers (19) figure *P. herbarum* bearing both Alternaria and sarcinaeform spores on the same hyphae. Gibelli and Griffini (8), Mattirolo (13), Bauk (1), and Kohl (12), studying *P. herbarum* in pure culture, concluded that it should be divided into two varieties or species, one having Alternaria conidia and the other having sarcinaeform conidia. Miyake (14), studying the life history of *Macrosporium parasiticum* Thum., found no Alternaria stage in the life cycle. Halsted (9), in studying the life history of *Pleospora tropaeoli* Hal. in pure culture, found that the cycle included only Pleospora and Alternaria stages.

As the ascigerous stage of most species of Alternaria and Macrosporium is unknown or non-existent, the basis for the distinction of genera and species must rest, in general, on the conidia. Jones (10–11), in studying *Macrosporium solani* E. & M. and *M. fasciculatum* C. & E.

on artificial media, found that they formed chains of conidia and placed them in the genus Alternaria. Bioletti (3) reported securing pure cultures of *Macrosporium* sp. and *Alternaria* sp. from olives in California. Others have reported isolating species of one or the other genera from various sources and growing them in pure culture under conditions where the morphology of the fungi ought to have been pretty certainly ascertained, but by far the greatest amount of literature on the two genera deals with their pathogenic effects or with the mere description of species.

Miyake (14), Prillieux and Delacroix, (17), and others (4) have shown by careful experiments or have suggested that many of the specific names are synonyms. Constantin (4) and Planchon (16) have reported great variations in Alternaria due to growth on different media. Planchon (16) expresses the opinion that Macrosporium is merely Alternaria with dissociated conidia. No extensive comparative work, either between the two genera or between species of the two genera, has yet been undertaken.

III. METHODS IN GENERAL

To aid in the comparison of published descriptions, a tabulation of the species and varieties of Alternaria and Macrosporium given in the "Sylloge Fungorum" was made on the basis of spore length, the species being arranged according to the maximum length given. Specimens from all available exsiccati were studied and compared. Cultures of the principal types found in the exsiccati were studied under varied conditions in order to learn something of the constancy of the characters which are made the basis of specific distinctions. The original generic descriptions were studied in order to ascertain the basis of generic distinction.

The Value of Written Descriptions

An examination of the specific descriptions of the two genera in question showed that spore measurements were most constantly used in distinguishing between species; in many cases several species were alike in every character given except that of size of spores. In order to learn something of the variation in measurements which may be due to the personal element and to the use of different micrometers and microscopes in measuring, three slides were prepared and together with cultures of *Phoma destructiva* Pat. were sent to a number of persons

actively engaged in descriptive mycological work. On one slide of *Pleurosigma angulatum* Sm., a single frustule was enclosed in a circle and indicated; on a second slide two spores of *Alternaria fasciculata* were similarly indicated. A third slide of *A. fasciculata* contained several hundred spores. Identical typewritten directions were sent to each one who made the measurements, asking that no more care be used than would ordinarily be taken in measuring for the purpose of describing a new species. A tabulation of results follows:

TABLE A.

Observer No.	Eyepiece Value	Single Frustule of *P. angulatum*	One Spore of Alternaria	One Spore of Alternaria	Many Spores of Alternaria	Many Spores of Phoma
1	2.4	285.6×45.6	48 ×7.2	19.2×9.6	12–36×6–12	2–5×1–2
2	2.4	284.4×46.8	49.2×6	19.2×9.6	14–36×9–12	3–4×2–3
3	1.8	284.4×45.9	50.6×7.2	19.8×9.9	11–41×6–13	
4	3.16	282 ×46.5	48 ×7.5	19.5×9.5	10.5–24×7.5–13	5–6×1.5–2
5	3.4	282 ×46	48 ×7	20 ×10	17–34×7–10	
6	3.2	280 ×45	48 ×7	20 ×7	13–29×9–11	4–8×2–3.5
7	4.	280 ×44	48 ×7	20 ×9	20×9	5–6×2–2.5
8	3.2	276 ×45	48 ×8	21 ×7	12–35×9–12	5–9×2–3
9	10.	275 ×50	.....	.....	13–35×7–11	.....
10 ...	3.2	275 ×45	47 ×7	20 ×10	12–33×6–12	3–8×2–3
11 ...	3.	270 ×48	50 ×7.5	21 ×9.5	15–40×8–12	5–8×3–5
Variation ...		15.6×6	3.6×2	1.8×3	6.5–17×3–3	3–4×2–3
Variation %.		5 ×12	7 ×25	8.5×30	43–41×33–23	60–44×66–60

The second column gives the value of the smallest division of each eyepiece micrometer in micromillimeters. All the measurements given in the table are in microns. The arrangement is according to the maximum measurement given for *Pleurosigma angulatum*.

The third, fourth and fifth columns show the variations in measurements when all, without any doubt, were measuring the same things. The variation is least in the longest measurements, being a little over 5 percent in the greatest length given, and greatest in one of the two shortest measurements, amounting to 30 percent in the width of the spore indicated in the fifth column. That this variation is not due to the eyepiece used is shown by the fact that observer No. 10 who returned the lowest measurement for the long spore, column *4*, also returned the highest measurement for the width of the spore given in column *5*. The variations given for the Phoma spores are the greatest, being 66 percent for the shortest measurement and 44 percent for the longest measurement. In this case each mycologist made his own microscopic preparation.

The most instructive results appear in the sixth column of the table where the maximum and minimum lengths and breadths of the spores are given under conditions such as would obtain in describing a new species. Here there was a variation of over 41 percent of the highest maximum measurement returned for the length of the spores. The variation for the minimum lengths was greater. Assuming variation equal to that shown in column *6* of Table A, 34 species of Macrosporium and 5 species of Alternaria in the "Sylloge Fungorum" are inseparable by measurements of both length and breadth.

Applying to all the species of Alternaria and Macrosporium in the "Sylloge Fungorum" the variability shown in column *6* of Table A, they can be combined into thirteen groups, taking into consideration the measurements for both length and breadth of spores. In other words, if other characters are disregarded, in so far as actual spore measurements are dependable, there are only thirteen species of Macrosporium and Alternaria adequately described in the "Sylloge Fungorum."

Study of Exsiccati

Following the study of descriptions in the "Sylloge Fungorum," examination was made of the specimens of Macrosporium and Alternaria in the exsiccati immediately available. One hundred and thirty-four specimens labeled as 85 species, were found. Of this number 17 were marked "sp. n." *i. e.*, of or nearly of the value of type material. Eighteen other species not marked "sp. n." were found in the exsiccati of the authors or one of the joint authors of the species. This gave a total of 35 species, the material of which can be regarded as reasonably authentic.

Mounts were made from each of the specimens, from which spore and conidiophore measurements, and the character of each, were recorded. The nature of the growth, whether apparently parasitic or saprophytic, was also recorded and the descriptions so made were compared. There was no doubt that in the collection, many specimens morphologically indistinguishable appeared under different names, and that in some instances the same name was given to specimens which were in no way similar or which could readily be distinguished from each other.

In the following summary of the study of the exsiccati the names are given as they appeared on the specimen, followed by the title of

the collection and the specimen number in the collection. Two asterisks (**) following the species name indicates that the specimen was marked "sp. n."; a single asterisk (*) indicates that the specimen was found in the exsiccati of the author of the species.

Group 1

The following species had only globular or packet-shaped spores and were essentially alike:

*Macrosporium sarcinaeforme** Cavara, Fungi Par., Bri. & Cav., 116.
M. cladosporioides Desm., Fungi Sel. Ex., Roum., 5596.
M. stilbosporoideum Bri. & Cav., N. Amer. Fungi, Ellis, 2080.

Group 2

A second group was made of those having globular or packet-shaped spores like those of the first group, but having in addition some ovate or pointed spores which might be due to variation in the shape of spore or to a mixture of two forms:

*M. parasiticum*** Thum., Myc. Univ. Thum., 667; Fungi Par., Bri. & Cav., 152.
*M. consortiale*** Thum., Myc. Univ. Thum., 1373.
M. sarcinula Berk., Fungi Columb., 3032.
M. chartarum Pk., N. Amer. Fungi, Ellis, 648; Fungi Sel. Ex,, Roum., 6560.
M. heteronemum (Desm.) Sacc. Fungi Sel. Ex., Roum., 6647, 6562, 6358.

The following were possibly the same as the above but they either showed minor differences or else the material was not sufficient to afford positive judgment:

M. chartarum Pk., Fungi Columb., 396.
M. zimmermaneii Thum., Fungi Sel. Ex., Roum., 396.
M. polytrichum Cke. & Rav., Fungi Par., Bri. & Cav., 191.
M. puccinioides E. &. And., N. Amer. Fungi, Ellis, 2876.

Group 3

A third group was made of species having long, narrow, regular, tapering spores with few longitudinal septa. All were apparently parasitic:

*M. euphorbiae*** Bart., Fungi Columb., 2633.
*M. carotae** E. &. E., N. Amer. Fungi, Ellis, 3289; Fungi Columb., 2632.

M. amaranthi Pk., Fungi Columb., 2631.
M. brassicae f. solani Faut. et Brun., Fungi Sel. Ex., Roum., 6559.
M. cheiranthi Fr., Fungi Amer., Rav., 303.
M. solani E. & M., Fungi Columb., 891; 398; 3635; Fungi. Par., Bri. & Cav., 191; Economic Fungi, Sey. & Earle, 339; 340; N. Amer. Fungi, Ellis, 1265; 2485.
Alternaria solani (E. & M.) J. & G., Econ. Fungi, Sey. & Earle, 521.
A. brassicae (Berk.) Sacc., Fungi Par., Bri. & Cav., 87.

Two forms similar to the above were:

*M. caudatum** C. & E., Fungi Columb., 397. This was like the above but uniformly shorter.
M. antennaeforme B. & C., Fungi. Columb., 2531. This species was in general form similar to those in the above group but the spores were uniformly more slender.

Group 4

Three other specimens were found with spores of the same form as the above group but the spores were much larger. These were:

*M. herculeum** E. &. M., N. Amer. Fungi, Ellis, 1263.
*M. commune** Rabh., Fungi Europ., Rabh., 1360.
M. saponariae Pk., N. Y. Fungi, Shear, 397; Fungi Sel. Ex., Roum., 3868.

Group 5

Another group was formed of species with spores similar to those of *Alternaria solani* but generally wider and shorter and always more markedly muriform. The species in this group are not morphologically identical but merely similar.

*M. heteroschemon*** Faut., Fungi Sel. Ex., Roum., 6942.
*M. cucumerinum** E. &. E., N. Amer. Fungi, Ellis, 3396.
M. nervii Cke., N. Amer. Fungi, Ellis, 964.
M. sucaviae Trabut., Fungi Sel. Ex., Roum., 4098.
M. convallariae Fr., Fungi Sel. Ex., Roum., 1897.
M. brassicae Berk., Fungi Sel. Ex., Roum., 6442.
M. martindalei E. &. M., N. Amer. Fungi, Ellis, 1262; Fungi Europ., Rabh., 3282.
M. cheiranthi Fr., Fungi Sel. Ex., Roum., 7235.
*Alternaria malvae** Roum., Fungi. Sel. Ex., Roum., 3393.
A. brassicae (Berk.) Sacc., Econ. Fungi, Sey. & Earle, 515.

Group 6

In the following group the specimens were essentially all alike; quite variable in color, shape, and size. The color varied from light to dark olive both in spores and conidiophores. Almost all appeared to be growing saprophytically.

*M. hibiscinum*** Thum., Myc. Uni., Thum., 979.

*M. baptisiae*** Thum., Myc. Uni., Thum., 1271; Fungi Sel. Ex., Roum., 4897.

*M. cassiaecolim*** Thum., Myc. Uni., Thum., 1270; Fungi Sel. Ex., Roum., 4795.

*M. gossypinum*** Thum., Myc. Uni., Thum., 1469; Fungi Sel. Ex., Roum., 4898.

*M. ravenelii*** Thum., Myc. Uni., Thum., 2071; Fungi Sel. Ex., Roum., 4680.

*M. rubi*** Ellis, N. Amer. Fungi, Ellis, 544.

*M. truncatum*** Laub. & Faut., Fungi, Gallici, Roum., 6752.

*M. inquinans** C. & E., N. Amer. Fungi, Ellis, 369.

*M. ornatissimum** E. & B., Fungi Columb., 1741.

*M. porri** C. & E., Fungi Columb., 1279; N. Amer. Fungi, Ellis, 370.

*M. caudatum** C. & E., Fungi, Amer., Rav., 607; Fungi Columb., 890; Fungi Columb., 397; N. Amer. Fungi, Ellis, 816.

*M. iridis** C. & E., N. Amer. Fungi, Ellis, 51.

*M. canificans** Thum., inid, Myc. Uni. Thum., 2280; Fungi. Gall. Ex., Roum., 4794.

*M. leguminum** Cke., Fungi Amer., Rav., 300; Fungi Amer., Rav., 603.

*M. maydis** C. & E., N. Amer. Fungi, Ellis, 3098; Rabh.-Winter, Fungi Europ., 3592.

*M. catalpae** E. & M., N. Amer. Fungi Ellis, 1264; Econ. Fungi, Sey. & Earle, 144.

*M. martindalei** E. &. M., N. Amer. Fungi, Ellis, 1262.

M. tomato Cke.,* N. Amer. Fungi, Ellis, 2484; Fungi Amer., Rav., 603.

M. tenuissimum Fr., Myc. Uni., Thum., 980.

M. convallariae Fr., Myc. Uni., Thum., 1965.

M. chartarum Pk., Fungi Columb., 396.

M. floridanum Cke., Fungi Amer., Rav., 299.

M. florigenum Ell. & Dear., N. Amer. Fungi, Ellis, 3097.

M. togenariae Thum., Fungi Columb., 1367.

M. clematis Pk., Fungi Columb., 1830.

M. bulbotrichum Cke., Fungi Amer., Rav., 604.

M. erumpens Cke., Fungi, Amer., Rav., 605.
M. graminum Cke., Fungi Amer., Rav., 606.
M. caespitulus Cke., Fungi Amer., Rav., 906.
M. cheiranthi Cke., Fungi Sel. Ex., Roum., 4490.
M. consortiale Thum., Fungi Sel. Ex., Roum., 4992.
M. saponiariae Pk., Fungi Sel. Ex., Roum., 3868.
M. phomoides Thum., Fungi Sel. Ex., Roum., 6145.
M. caespitulorum Rabh., Fungi Sel. Ex., Roum., 7236.
M. fasciculatum C. & E., Fungi Sel. Ex., Roum., 1058; N. Amer., Fungi, Ellis, 52; Fungi, Columb., 399; Myc. Uni., Thum., 1870.
M. commune Rabh., Fungi Sel. Ex., Roum., 2068, 4239, 3288, 6443; Fungi Amer., Rav., 304; Fungi Columb., 2330; N. Amer. Fungi, Ellis, 418.
Alternaria fasciculata (C. & E.) J. & G., Econ. Fungi, Sey. & Earle, 522; Fungi Columb., 1368.

Except for being lighter colored, *M. peponicolum*** Rabh., Fungi Europ., Rabh., 1285, was like those in the above group. Several others were in most particulars like the above but either differed in some respects or else the material was too scanty for judgment. These were:

*M. abruptum** C. & E., N. Amer. Fungi, Ellis, 127; Fungi Amer., Rav., 302.
*M. phaseoli*** Faut., Fungi Sel. Ex., Roum., 6247.
*M. cercosporoides** C. &. E., Fungi Columb., 1740.
*M. valerianellae** Roum., Fungi Sel. Ex., Roum., 3690.
*M. elegantissimum** Rabh., Fungi Europ., 2883; Fungi Sel. Ex., Roum., 2067.
M. concinum B. & Br., Fungi Sel. Ex., Roum., 6443.
M. commune Rabh., Fungi. Sel. Ex., Roum., 4240.
M. puccinioides E. & And., Fungi Columb., 1172.

Group 7

Two quite similar species which differed from any other specimens were:

*M. junci*** Lamb. & Faut., Fungi Sel. Ex., Roum., 6444.
M. brassicae Berk., Fungi Sel. Ex., Roum., 2363; N. Amer. Fungi, Ellis, 2483.

They were like *Alternaria brassicae* var. *microspora*, which the latter undoubtedly was.

One species, *A. cucurbitae*** Let. & Roum., Fungi Sel. Ex., Roum., 3694, did not afford enough material for judgment.

The above study of exsiccati and descriptions brings not only species into question but genera as well, since in all but the first and second of the above groups both Alternaria and Macrosporium are included in groups as morphologically similar.

IV. EXPERIMENTAL RESULTS

Cultures of Alternaria and Macrosporium and material upon which either was growing were secured from many sources.[1] Of eighty cultures thus obtained all but two produced chains of spores regularly on artificial media and accordingly belonged in the genus Alternaria. All of these had clavate, elongate or ovate, more or less pointed spores. The two which did not ordinarily produce chains of spores had globular or sarcinaeform conidia. One of these very rarely produced chains of two spores, in which cases the bottom spore was pointed. Eleven of the cultures were selected as representative of all the forms present and as most suitable for extensive study. These eleven cultures also represented all of the morphological forms found in exsiccati. They were: *Alternaria solani* (E. & M.) J. & G., isolated from blighted potato leaves (*Solanum tuberosum* L.); *A. solani* isolated from Datura leaf spot (*Datura stramonium* L.); *A. brassicae* var. *nigrescens* Peglion, isolated from blighted cantaloupe leaves (*Cucumis melo* L.); *A. bras-*

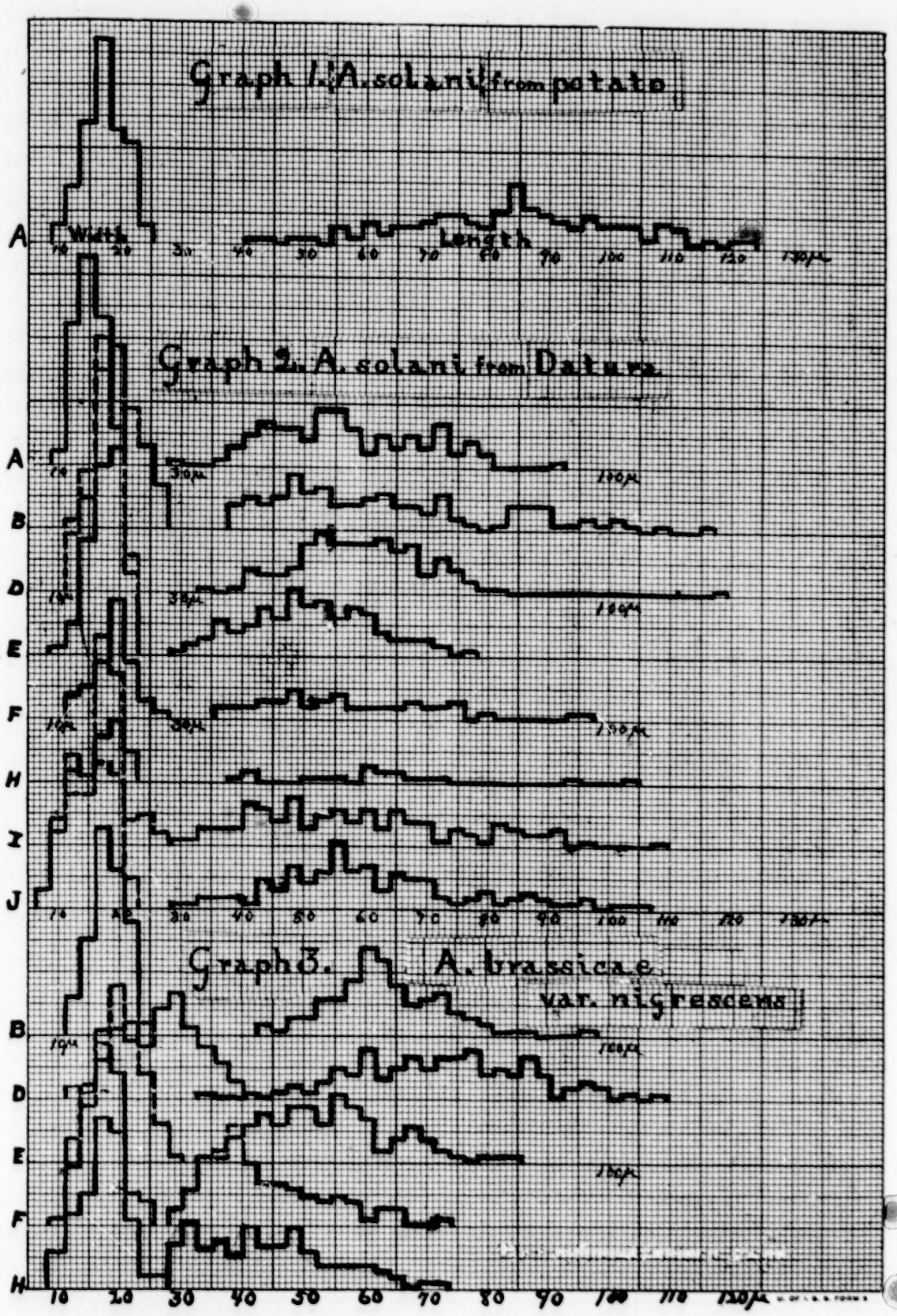
EXPLANATION OF GRAPHS 1 TO 9

The measurements of spores are indicated in microns by the base line, each space representing one μ. The frequency is indicated on the perpendicular lines, each space representing one spore. Measurements are at intervals of 2.4 μ except in the case of the narrower spores where the width is taken at intervals of 1.2 μ.

The following letters are used to indicate the host and media: *A*, natural host; *B*, bean agar, 30°; *C*, bean agar, 10°; *D*, bean agar, + 20 Fuller's scale; *E*, bean agar, − 20 Fuller's scale; *F*, synthetic agar; *G*, synthetic agar minus glucose; *H*, synthetic agar with double amount of glucose; *I*, synthetic agar with double amount of asparagin; *J*, synthetic agar without asparagin; *K*, leached agar.

The most striking facts to be observed are: general reduction in size of spores on the synthetic agars (*F* to *J*), over that on bean agars (*B* to *E*). Relative constancy of species given in graphs 2, 4, 5, and 6 over those given in graphs 3, 7, 8, and 9. Increase in size of spores at lower temperature (Graphs 7 and 9; *C*). Extreme range of variation in size of larger spores (graphs 1, 2, 3, 8 and 9).

[1] Cultures or material was received from J. J. Davis, L. R. Jones, B. F. Lutman, W. G. Sackett, S. M. Tracy, H. T. Güssow, E. C. Stakman, G. P. Clinton, M. T. Cook, G. F. Atkinson, B. D. Halsted, I. M. Lewis, C. W. Edgerton, C. R. Orton, B. B. Higgins, J. W. Eastham, Miss Jean MacInnes, G. L. Peltier, F. C. Stewart.


Graph 1. A. solani from potato
A
Width
Length
Graph 2. A. solani from Datura
A
B
D
E
F
H
I
J
Graph 3. A. brassicae var. nigrescens
B
D
E
F
H
10 20 30 40 50 60 70 80 90 100 110 120µ

sicae var. *microspora* (Berk.), Sacc., isolated from cabbage leaf spot (*Brassica oleracea* L.); *A. fasciculata* (C. & E.) J. & G., isolated from potato leaf spot; *A. tenuis* Nees, isolated from decaying wood; *A. dianthi* Stev. & Hall, isolated from Dianthus leaf spot; *A. iridicola* (E. & E.) n. com. (*Macrosporium iridicolum* E. & E.), isolated from iris leaf spot (*Iris germanica* L.); *A. sonchi* Davis, isolated from leaf spot of *Sonchus asper* (L.) Hill; *Macrosporium sarcinaeforme* Cavara, isolated from clover leaf spot (*Trifolium pratense* L.); *M. sarcinula* Berk., isolated from decayed spot on Jonathan apple (*Pyrus malus* L.).

All studies were made with pure cultures originating from a single spore of each species used, the spores being located on thin poured plates and transferred before germinating to other plates. The instruments invented by Keitt[1] greatly facilitated this operation.

Whenever it was possible to do so, ten-day-old cultures were used as the standard for comparisons. Measurements of spores and conidiophores were made whenever present. In measuring spores a mechanical stage was used and every spore on the slide which came within the field was measured for both length and width, thus eliminating unconscious selection. Curves of the measurements of each hundred spores were made for each culture. Since in a given species there is frequent variation in the length of the beaks, from one third to six or seven times the length of the spores, the beaks were not in any case included in the measurements.

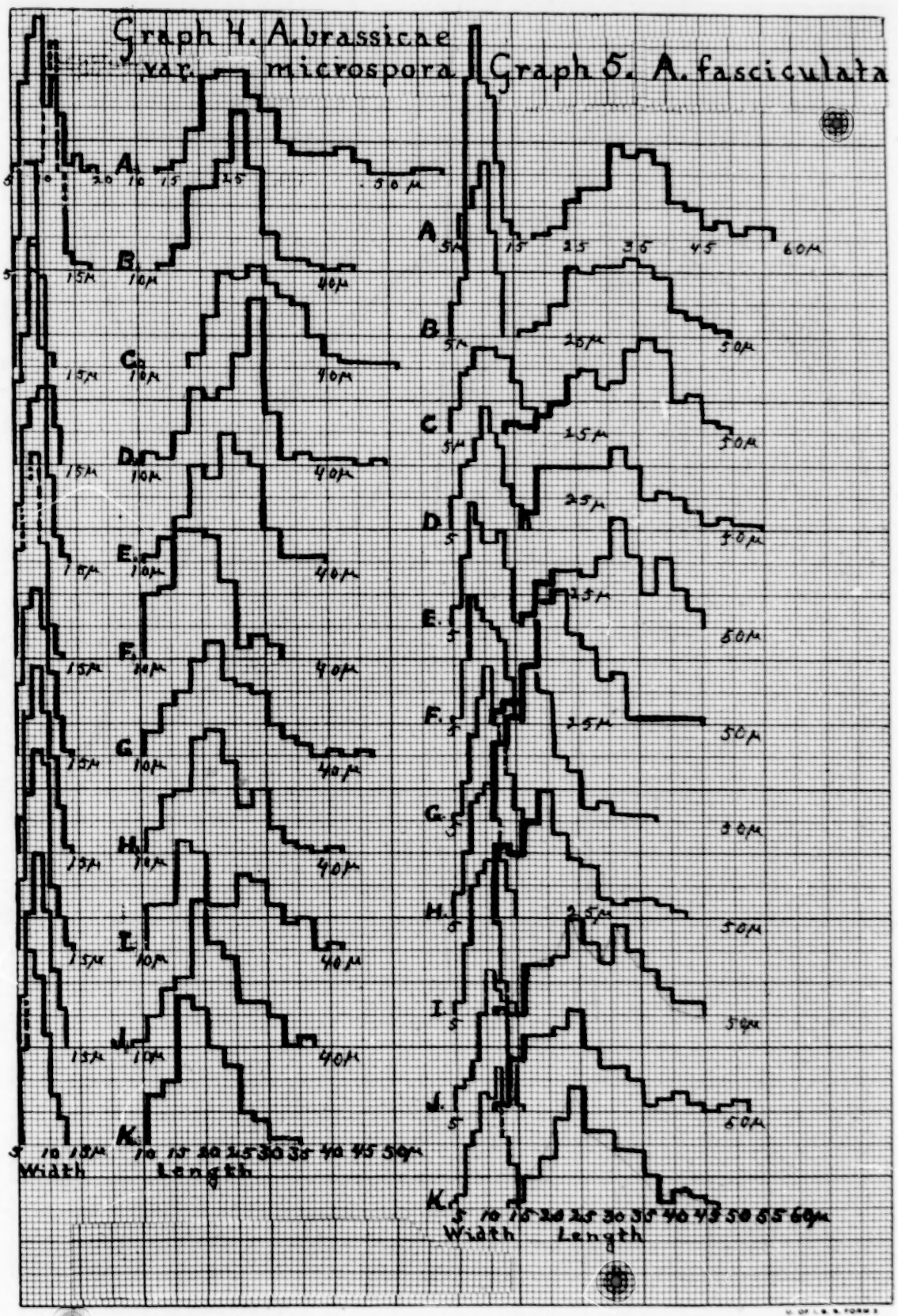
Graphs giving the superimposed curves of the measurements of one hundred spores of the same species under different conditions were prepared for the sake of easy comparison of the variations due to differences in cultural conditions (Graphs 1–9).

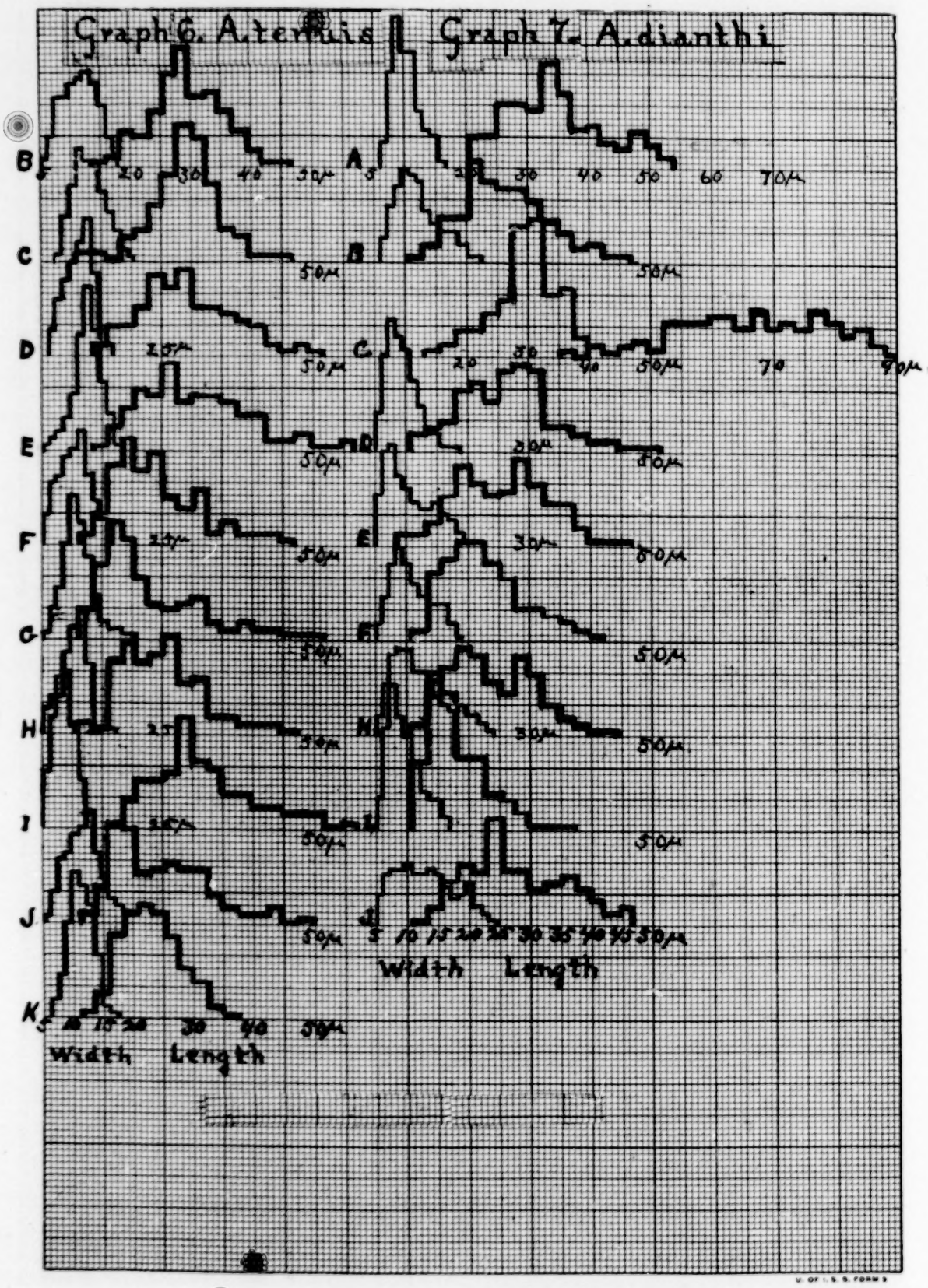
Host Relation and Parasitism

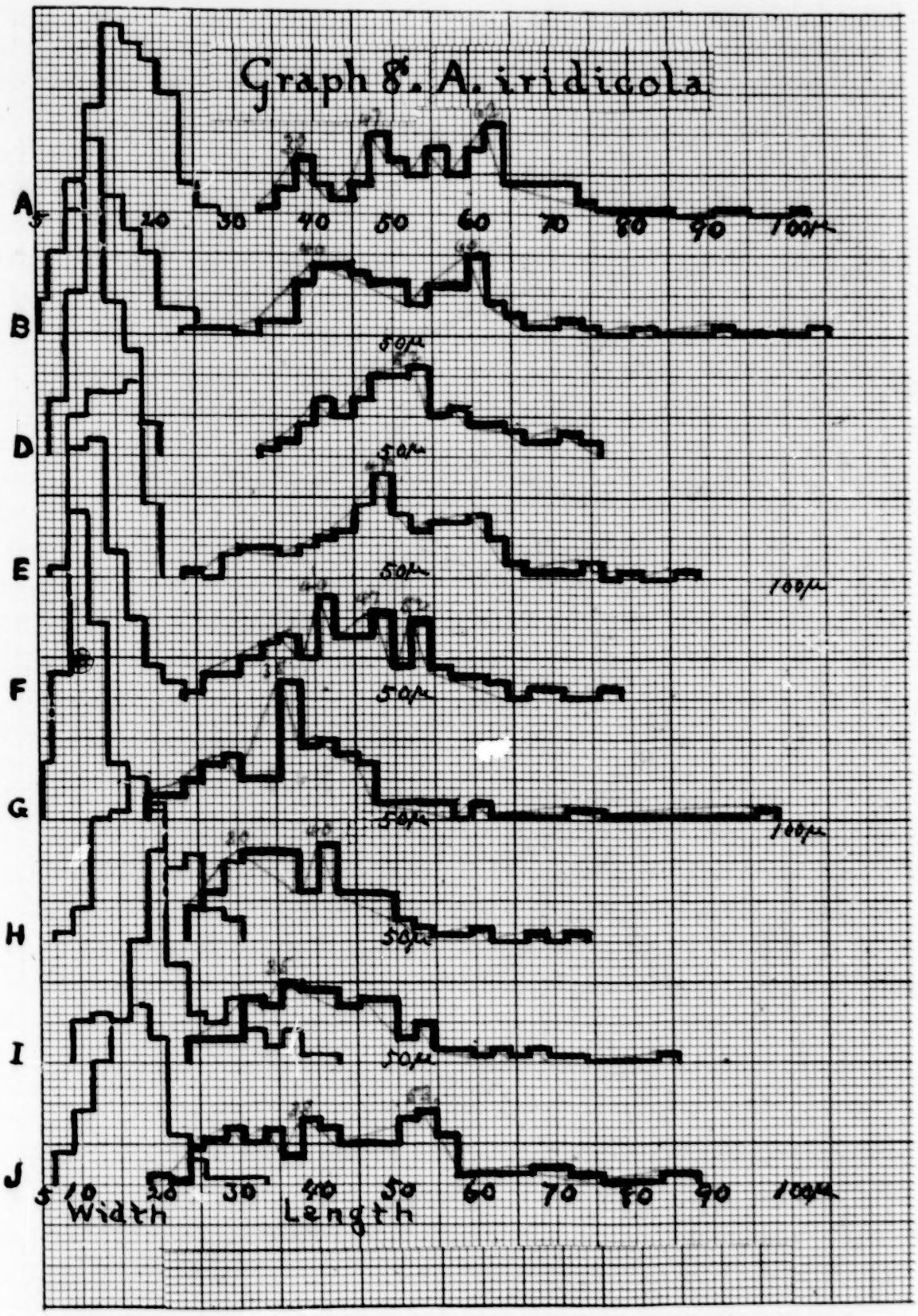
Inoculations were made on the natural hosts, on their near relatives, and on other plants, when there seemed to be any advantage in so doing. For example, *Alternaria solani*, which is morphologically similar to *A. brassicae*, was inoculated on cabbage leaves.

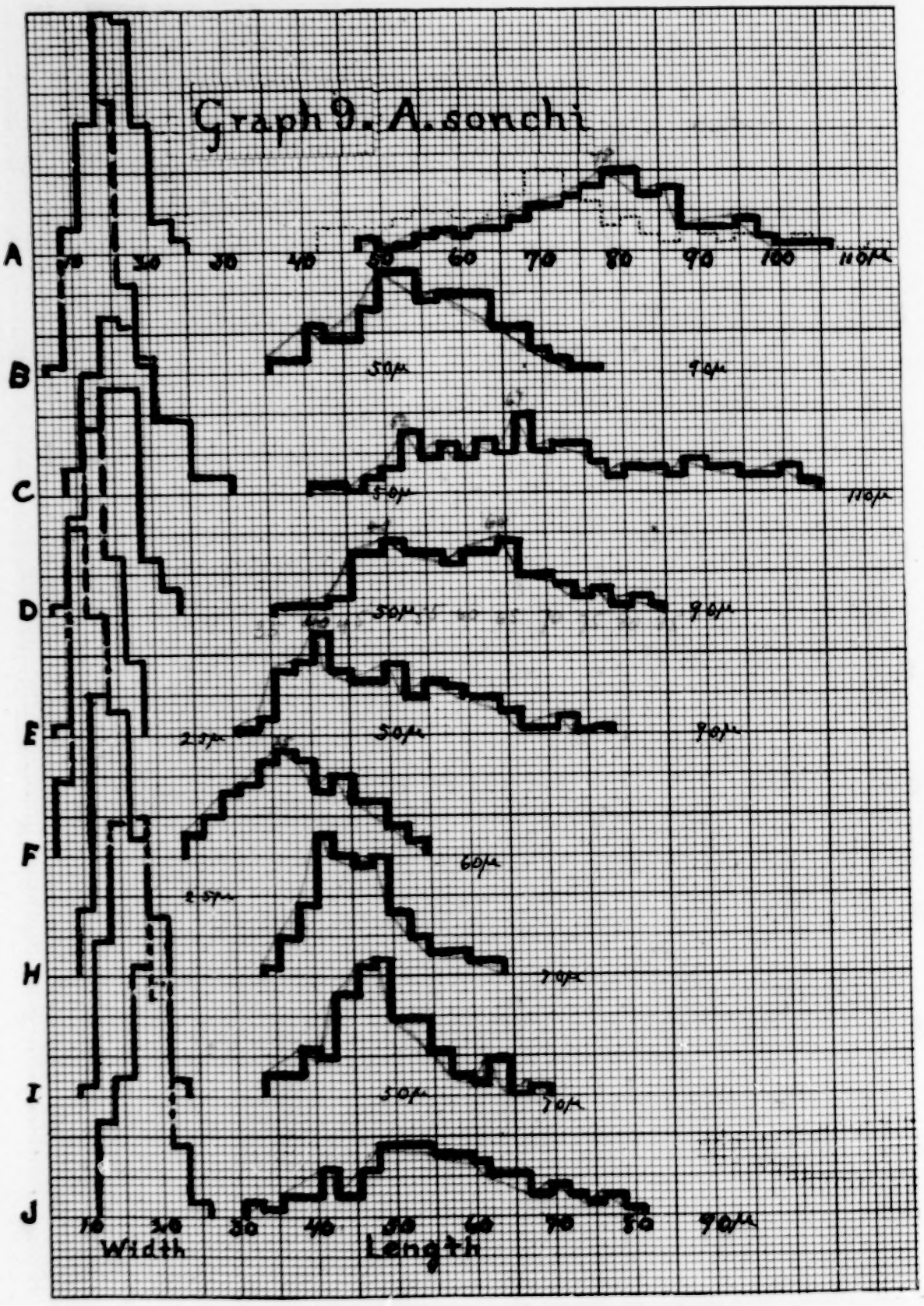
The plants were inoculated by needle pricks and by placing spores in drops of water on the unbroken leaf surface. The percentage of successful inoculations was estimated from the needle pricks, since these were more easily located. An inoculation was considered successful if the fungus to a notable degree invaded and killed the tissues

[1] Phytopathology **5**: 266, 1915.


Graph 4. A. brassicae var. microspora
Graph 5. A. fasciculata
Width
Length


Graph 6. A. tenuis
Graph 7. A. dianthi
Width
Length
Width
Length


Graph 8. A. iridicola
A
B
D
E
F
G
H
I
J
5 10 20 30 40 50 60 70 80 90 100μ
Width
Length


Graph 9. A. sonchi
A
B
C
D
E
F
H
I
J
Width
Length

surrounding the punctures. Unless otherwise stated, successful inoculations also occurred on the unbroken leaf surface. To check the results of the needle-prick inoculations, sterile needle pricks were always made and all the plants kept under identical conditions. In no case did the control plants show signs of fungous invasion around the needle pricks. They will receive no further mention. Unless otherwise stated, spores of the fungus used were always re-isolated from the spots caused by the inoculations. In the following discussion the terms "normal" and "characteristic" indicate that the spot or fungus appeared the same as in the cases of natural infection.

Alternaria solani from potato leaves: Inoculations were made from the first and tenth generations on agar and from spores taken directly from potato leaf spots. The mycelium was used as the inoculum, since very few spores were produced in culture. All of the inoculations with mycelium were made by needle pricks on plants under bell jars. The plants inoculated were: potato (*Solanum tuberosum); Datura stramonium;* tomato (*Lycopersicum esculentum* [L.] Mill.) leaves and fruit; *Solanum nigrum* L.; cabbage (*Brassicae oleracea* L.); iris (*Iris germanica* L.); *Lactuca canadensis* L.

All inoculations on solanaceous hosts were successful; about 50 percent of the inoculations on cabbage leaves failed; none succeeded on iris or Lactuca. The development of the spots was less rapid on Datura than on any other of the solanaceous hosts. Spots were all of the characteristic "target board" type. Very few spores were produced on any of the spots—none on the cabbage leaf.

A. solani from Datura leaves: Inoculations by needle pricks and on the uninjured leaf surface were made with spores taken directly from leaf spots and with spores of the first and tenth generations from agar plates. All plants were kept under bell jars. The same plants were used as in inoculations with the strain from potato, and the results were similar except that all attempted inoculations on cabbage failed. The fungus developed least vigorously on potato leaves. On the other solanaceous hosts the spots were not different from those caused by *A. solani* from potato. Very few spores were produced on any of the spots.

A. brassicae var. *nigrescens* from cantaloupe leaves: Inoculations were made with small pieces of diseased tissue, also with spores from the first, sixth and tenth generations on agar, by means of needle pricks and on the uninjured leaf surface. The plants were kept under bell

jars for the first few days following inoculations. Inoculations were attempted on cantaloupe (*Cucumis melo*), cabbage, Datura, and Lactuca. Characteristic spots were produced on cantaloupe in all cases; 25 percent of the inoculations on cabbage were successful; no spots were formed on Lactuca or Datura.

The spots produced on cantaloupe leaves, appearing in 2 or 3 days, developed rapidly. The spots on cabbage leaves were mere dots where the spores had germinated on the unbroken leaf surface, but of considerable size where punctures were made. No spores were produced on cabbage and very few on cantaloupe leaves.

A. brassicae var. *microspora* from cabbage leaf spots: All inoculations were made with spores from pure cultures, by needle pricks or on the unbroken leaf surface. Inoculations were attempted on cabbage, radish (*Raphanus sativus* L.), *Lobularia maritima* (L.) Desv., Dianthus, potato, Datura, tomato leaves and fruit. On all cruciferous plants the fungus produced characteristic concentric spots with dark rings of spores on the surface. Spores spread the fungus to other parts of susceptible plants until they were entirely destroyed. On ripe tomatoes dark spots were formed with narrow sharply defined zones of spores. No spots were formed on the other plants used.

A. fasciculata from potato leaf spots: Inoculations were made with spores by means of needle pricks and on the unbroken leaf surface. The plants inoculated were: potato, tomato leaves and fruit, cabbage, radish, Dianthus.

The only definite spots of parasitic appearance were from needle pricks on etiolated or partially etiolated cabbage leaves. The spores and conidiophores on the cabbage leaf spots were very light amber in color, instead of dark olive as is normal. In ripe tomatoes a hard black core was formed in the interior as the mycelium invaded the tissues. This was surrounded by a soft decayed area. The inoculations failed on the other plants.

A. tenuis from decaying wood: Only one series of inoculations was attempted with this species. This was made on Dianthus leaves by means of needle pricks. No spots were formed. The inoculations were made on Dianthus because *A. tenuis* spores cannot be distinguished from those of *A. dianthi*.

A. dianthi from Dianthus leaf spots: Inoculations were made both by needle pricks and on unwounded leaf surface with spores of the first and fifth generations from agar plates. Dianthus and cabbage

were the only plants used. No spots were formed on cabbage leaves. All inoculations on Dianthus were successful. In air of natural humidity the spots remained very small but under a bell jar they spread rapidly, produced concentric rings of dark spores on the surface and finally killed the plants.

A. iridicola from iris leaf spots: Inoculations were made entirely by means of needle pricks. Both spores and mycelium were used. The plants inoculated were: Iris, *Lilium philadelphicum* L., Datura, potato, cabbage, Lactuca, cantaloupe, and onion (*Allium cepa* L.). All inoculations except those on iris and onion failed. Spots on iris extended slowly, on onion tops more rapidly, accompanied by the production of a considerable number of spores. Inoculations were made on Datura, potato, cabbage, and cantaloupe because of the general similarity of the spores of *A. iridicola* and *A. solani* (Plate XX, Fig. 7).

A. sonchi from leaf spots on *Sonchus asper:* The fungus could not be isolated from the spots on *Sonchus asper* since the spores would not germinate in culture. Crude inoculations were made on *Lactuca canadensis*, using as the inoculum diseased leaf fragments of *S. asper*. These inoculations were successful and the production of spores on *L. canadensis* abundant. Pure cultures were obtained from *L. canadensis* leaf spots. Inoculations were made by needle pricks and on the unbroken leaf surface. Inoculations were made on *Lactuca canadensis*, *L. sativa* L., *Taraxacum officinale* Weber, cabbage, tomato, and Datura. Spots were formed only on the Compositae. On *L. canadensis* very rapidly spreading characteristic dark brown spots were formed in two days. On cultivated lettuce and dandelion, distinct spots were formed around needle-prick inoculations, but soon ceased to grow and no spores were produced. Spore production was abundant on *L. canadensis*. (Plate XX, Fig. 8).

Macrosporium sarcinaeforme from leaf spots on red clover: Inoculations were made with spores, both on the unwounded leaf surface and by needle pricks. Red clover (*Trifolium pratense*), white clover (*T. repens* L.), alfalfa (*Medicago sativa* L.), cow pea (*Vigna sinensis* Endl.), *Cucumis melo*, and Allium, were inoculated. The fungus was actively parasitic only on the clovers and alfalfa. Spots developed most rapidly on red clover and spores scattered the disease over the entire plant. On white clover and alfalfa the spots did not spread to leaves that had not been inoculated. In all cases the spots appeared within three days of inoculation. On onion, small white spots were

occasionally produced around needle pricks but no spores were produced and the parasitism was evidently very feeble.

M. sarcinula from apple fruit spot: This fungus was isolated by Miss Jean MacInnes, of the University of Illinois, in her studies of the rots of apples.[3] It appeared to be causing an apple rot. Small black pycnidia-like bodies were scattered frequently over the rotted area under the epidermis of the apple. These also appeared in the first generation on agar. When broken they appeared to be merely sclerotia. Spores from pure cultures were used in making inoculations both by needle pricks and on uninjured leaf surface. The fungus conformed to descriptions and figures of *M. sarcinula*. The same plants were inoculated with *M. sarcinula* as with *M. sarcinaeforme*. Spots were produced only on red clover and alfalfa. All attempts to inoculate apples failed. The spots on red clover were lighter colored but otherwise almost identical with those caused by *M. sarciniaeforme*. Few spores were produced on the spots. On the onion tops the fungus produced small white spots surrounding the punctures but no spores were formed.

The most noteworthy results of the inoculation experiments occurred in respect to *A. solani*, *A. sonchi*, *A. brassicae* var. *nigrescens*, and *M. sarcinula*. Both strains of *A. solani* grew freely on all of the solanaceous hosts, and one strain was feebly parasitic on cabbage. Morphologically, judging from exsiccati, *A. solani* and *A. brassicae* are identical, and the inoculation experiments might be considered further evidence of this view. *A. sonchi* was actively parasitic on Lactuca, a near relative of Sonchus, and was possibly very feebly parasitic on other Compositae. *A. brassicae* var. *nigrescens* proved slightly parasitic on cabbage although this would hardly be expected from nearness of Cruciferae and Cucurbitaceae. *M. sarcinaeforme* and *M. sarcinula* are entirely dissimilar except in general form of spores, yet they appeared equally parasitic on red clover and alfalfa, and both were feebly parasitic on onion tops.

Cultures on Agar

For purposes of isolation and general study, standard lima bean agar (beans 100 g., agar 15 g., water 1,000 g.) was used. The cultures were kept in darkness at 30° C., with the exception that *A. sonchi*, which would not grow at 30°, was grown at 20°. In testing the effect

[3] Unpublished thesis.

of a lower temperature, cultures on standard bean agar were kept at 10° C. The effect of acidity, also alkalinity, was determined on standard bean agar, using 30 cc. and 20 cc. of normal hydrochloric acid, or 20 cc. of normal sodium carbonate per liter. A standard synthetic agar was used to study the effect of nutrition: 1.36 g. acid potassium phosphate, 1.06 g. sodium carbonate, .5 g. magnesium sulphate, 5 g. glucose, 1 g. asparagin, 15 g. agar, 1000 g. water. Variations from this standard were made by omitting glucose, doubling the amount of glucose, omitting asparagin, and by doubling the amount of asparagin. Plain agar, 15 g. per liter of water, washed for several days in distilled water, was also used.

Records of all cultures on the various media were made in tabular form, but for the sake of brevity only the table for cultures on bean agar is given here; the differences shown on other media being briefly summarized. Variations in size of spores are given on graphs one to nine.

The most striking characters brought out by the colonies on the standard bean agar were the wide differences in the two strains of *A. solani* which on their hosts are indistinguishable. The strain from potato produced a pure white colony with marked red chromogenesis in the medium, had straight colorless submerged mycelium and no spores. On account of the abundant production of conidiophores and spores the strain of *A. solani* from Datura formed a gray colony, it produced no chromogenesis, and the submerged mycelium was dark olive and torulose. *A. fasciculata*, *A. tenuis*, and *A. dianthi* produced spores indistinguishable from each other. The conidiophores of *A. dianthi* were slightly larger in cross section than those of the other two. In general appearance of the colonies and in the production of aerial mycelium these three species were different. The other Alternaria species studied were quite distinct in most cultural characters. The two species of Macrosporium were totally unlike except in general form of spores.

Bean agar, 10°, 30 days: Zonation was absent or inconspicuous in most of the colonies, especially in those of *A. brassicae* var. *microspora* which at 30° had the most marked zonation of all the species. This species also showed a marked alteration in color and form of its spores, these being light amber, nearly colorless, and about half the width of the normal dark olive spores. Another marked change occurred in the size and color of the spores of *A. dianthi*, these being twice their normal

TABLE B.

Fungus	Growth, Mm.	Zonation General Character	Aerial Mycelium	Submerged Mycelium	Conidiophores	Spores
Alternaria solani from potato	33	Pure white, wide zoned, medium colored brick red.	Abundant, long white, cottony.	Colorless, straight.	None	None
A. solani from Datura	33–35	Gray, wide zoned colony, no chromogenesis.	Abundant, long, white.	Dark olive, much branched and torulose.	Abundant, normal or much constricted.	Abundant, normal in size, but much constricted.
A. brassicae, V. nigrescens	42	Very dark, especially near the center	Very little, white.	Dark olive, straight.	Few, dark olive, normal.	Few, dark olive, normal.
A. brassicae, V. microspora	28–30	Black colony with narrow sharply defined zonation.	Very little, short, white, weak.	Colorless or nearly so, straight.	Abundant, light-olive, normal.	Very abundant, normal.
A. fasciculata	32	Gray, wide zoned.	Abundant, white, long.	Colorless, turning olive with age, straight.	Abundant, light olive.	Very abundant, normal.
A. tenuis	27	Dark in center, pale on outer edge, zonation well defined.	Little, white, long.	Colorless at first, turning olive, straight.	Not abundant, light olive.	Fairly abundant, normal.
A. dianthi	15–16	Gray, zoned colony.	Considerable, long, white.	Colorless, or very light olive, straight.	Abundant, light olive.	Abundant, normal.
A. iridicola	15–17	White or gray cottony, zonation ill defined.	Long, white, cottony.	Colorless, straight.	Fairly abundant, very pale olive.	Fairly abundant, normal, light olive.
A. sonchi	25, 20° C.	Pure white, cottony colony with dense mycelial zone, slight yellow chromogenesis.	Very abundant, white, cottony.	Colorless, straight.	In clumps, very pale olive, normal.	In clumps under mycelial zones, smaller than normal.
Macrosporium sarcinaeforme	10–12	Black, dense colony.	Very little, short, white, weak.	Dark olive, much branched and torulose	Abundant, dark, normal.	Abundant, dark olive, normal.
M. sarcinula	10–13	Cottony, white.	Abundant, long, white, cottony.	Colorless, straight.	Abundant, light olive, normal.	Abundant, olive, normal.

dimensions and of much lighter color (Plate XIX, 10). A marked change in the character of both the aerial and submerged mycelium occurred in *M. sarcinaeforme*, which is normally black, but here was white. All colonies were much retarded in rate of growth, the amount varying with the species. Five produced no spores.

Bean agar + 30 Fuller's scale, 30°, 10 days: This medium did not solidify and in many cases the whole colony was saturated with it. There was little variation in the general appearance of most of the colonies from that on standard bean agar. Two variations worthy of note were the absence of zonation in colonies of *A. brassicae* var. *microspora*, and the more than usual production of aerial mycelium on colonies of *A. brassicae* var. *nigrescens*. The chromogenesis of *A. solani* from potato was more marked on this than on any other medium. Some species which usually produced spores in more or less abundance produced few spores or none on this medium. They were *A. brassicae* var. *nigrescens*, *M. sarcinaeforme* and *M. sarcinula*. The spores of *A. sonchi* and *M. sarcinula* were below normal size.

Bean agar + 20 Fuller's scale, 30°, 10 days: Except for a little greater production of aerial mycelium by most species, the general appearance of the colonies was like those on neutral bean agar. Chromogenesis of *A. solani* was very marked, also the submerged mycelium of this species was darker than normal. *A. brassicae* var. *nigrescens*, *M. sarcinaeforme*, and *M. sarcinula* produced fewer spores than on neutral agar.

Bean agar − 20 Fuller's scale, 30°, 10 days: On the alkaline bean agar the most notable feature was the abundant production of spores by *A. brassicae* var. *nigrescens*, which ordinarily produced few spores on artificial media or on its natural host. In this instance the spores covered all parts of the colony. *A. solani* from potato, which on neutral and acid media produced marked chromogenesis, on the alkaline medium produced none, and the submerged mycelium, in the older part of the colonies, became dark. *A. dianthi* and *M. sarcinula* produced spores smaller than normal on the alkaline medium. The general appearance of the colonies was little different from that on neutral bean agar with the exceptions already noted.

Standard synthetic medium, 30°, 10 days: Except for a general lack of aerial mycelium and the appearance of less luxuriance, the colonies were little different from those on the standard lima bean agar.

Standard synthetic agar minus glucose, 30°, 10 days: The appearance

of the colonies on this medium was very similar to that on washed plain agar, although the appearance of starvation was not so emphasized.

Standard synthetic agar with double amount of glucose, 30°, 10 days: The aerial parts of the colonies, mycelium, conidiophores and spores, were more abundant than on the standard medium. The size of the spores of some species was much reduced (Graphs 4, 8, 9; Curve H).

Standard synthetic agar with double amount of asparagin, 30°, 10 days: All colonies appeared less luxuriant than on the standard synthetic agar or bean agar.

Standard synthetic agar minus asparagin, 30°, 10 days: Partial starvation appeared in all species, and the submerged mycelium was darkened in most cases. The latter was most noticeable in *A. solani* from potato which usually had colorless mycelium. This same fungus produced a few spores on this medium, which was also unusual. Aerial mycelium was generally very meager in all species.

Plain washed agar, 20°: But for the mycelium of *Macrosporium sarcinaeforme*, the only characters by which the different species could be identified were those of the spores and conidiophores which were natural (Plate XIX., 1, 3, 5, 7, 8, 11, 16, 17; XX: 7, 9). Aerial mycelium was lacking and the submerged mycelium colorless and without distinctive features. Spores and conidiophores were characteristic. This was the only artificial medium upon which *A. solani* from potato produced spores in any abundance. *A. solani*, from Datura, did not produce spores until after eighty days. The torulose mycelium characteristic of this strain on most media was scarcely noticeable on the washed agar. *Macrosporium sarcinaeforme* produced dark torulose submerged mycelium characteristic of the species.

Variations under Different Conditions of Humidity

The effect of humidity upon the colony characters was studied by keeping the cultures at 20° C. in saturated atmosphere; room humidity (variation 45–70 percent); and in an atmosphere kept dry by the use of calcium chloride. In order to prevent desiccation of the medium, all the plates were coated with a thin layer of paraffin. After the agar had hardened, hot sterile paraffin was poured over the agar and the excess poured off. This left a thin film of paraffin effectively protecting the medium from desiccation or contamination. The paraffin was punctured in several places by needle pricks and spores

of the fungi planted in one of the punctures. The cultures were kept with the plates inverted, without covers. In no case was there any contamination after the plates had been coated and very little occurred during the process. In the least humid atmosphere there appeared to be no change in the water content of the medium even after sixty days.

In all cases where aerial mycelium was produced, there was an increase in its production in the saturated atmosphere over that under ordinary conditions. Those species producing the greatest abundance of mycelium produced proportionately fewer spores. On account of the presence of the paraffin film, the usual colony form was lacking. *A. brassicae* var. *microspora* and *M. sarcinaeforme*, both of which produced almost no aerial mycelium, produced an abundance of spores. At room humidity little mycelium was produced, and that was in tufts around the needle pricks. The relative production of spores was greater than in the saturated atmosphere; the submerged mycelium grew rapidly under the paraffin and covered the plate. In some cases the conidiophores ruptured the paraffin coat. This was true of *M. sarcinaeforme* to a greater extent than of any other species. In the very dry atmosphere no aerial mycelium and no spores were produced with the exception that *M. sarcinaeforme* produced a few spores in air bubbles in the paraffin coating.

Reaction to Bacterial Influence

One species of bacterium which will be designated as *B. x*, occasionally occurred as a contamination on plates. It had a marked inhibitory action upon the growth of the fungi and was therefore used for special study. Plantings of all the species of fungi that were being studied were made in the usual way, then after four days the plates were inoculated on four sides at about 1 mm. from the outer edge of the colonies with cultures of *B. coli*, 2 strains, *B. subtilis*, and *B. x*.

In all cases the fungi grew through the colonies of *B. coli* without showing any reaction. Occasionally in colonies of *B. subtilis* there was slight darkening of the mycelium. On the sides of the fungous colonies opposed to the colonies of *B. x*, however, there was in every case a marked reaction by the submerged mycelium. The hyphae were inhibited greatly in growth, were much darkened and extremely torulose. Nodules were produced along the hyphal threads and at the extremities of hyphal branches. The reaction by all the fungi was

essentially the same. In all cases there was no resemblance between the normal and the abnormal mycelium (Plate XX: figs. 1–6). The aerial parts of the colonies did not show reaction to the bacterial influence.

Chromogensis on Rice

On account of the chromogenesis exhibited by two of the species, cultures were made on boiled rice (1 g. rice, 10 g. water autoclaved at 120° C. in test tubes). The colonies were allowed to grow for two months before comparisons were made, although it was noted that *A. solani* from potato, *A. sonchi* and *M. sarcinula* showed more or less marked chromogenesis within three or four days after inoculation.

The most marked variations were those between the two strains of *A. solani*, which throughout the other experiments had shown many differences in reaction to media. *A. solani* from potato colored the rice light orange-red at the bottom of the test tube, deep orange-red or brick-red at the lower limit of mycelial growth. The part of the rice through which the mycelium grew was dark-red-brown. The aerial mycelium was also red-brown. *A. solani* from Datura did not color the medium below the mycelium. The part of the medium occupied by the colony was dark gray or black. The aerial mycelium was white. *A sonchi* colored the medium lemon-yellow; *A. iridicola*, yellow-brown; *A. fasciculata*, *A. tenuis*, and *A. dianthi* light brown; *A. brassicae* var. *nigrescens* gave a very faint brown; *A. brassicae* var. *microspora* gave no coloration. The medium was colored deep pink by *Macrosporium sarcinaeforme;* blue-gray by *M. sarcinula*.

The remarkable thing about the reactions of these fungi to various media was that it was impossible to predict from the reaction of one species, what the reaction of another would be. A case in point was the reaction to alkaline and acid media. *A. brassicae* var. *nigrescens* showed a gradual increase in production of spores from high acid to alkaline media, the production in the latter case being very abundant. *A. solani*, from Datura, produced very few spores on the alkaline medium while on the acid media the spore production was abundant. Several species showed a lowered spore production in the acid media, notably those which normally produced few spores. *Macrosporium sarcinaeforme*, which usually produced spores in great abundance, produced none on the higher acid medium.

Lack of nutritive substances affected all species alike, causing a

general lack of luxuriance, lack of color in submerged mycelium and absence of aerial mycelium. This reached the extreme on the leached agar, where the aerial mycelium was almost entirely lacking and the submerged mycelium colorless and not at all characteristic, with the exception of *M. sarcinaeforme*.

The comparative studies noted above were made with cultures which were ten days old, but all of the cultures were maintained thirty days or longer—sometimes ninety days. In some cases marked variations due to age were noted in the spores. In several species secondary growth or development of spores occurred which entirely destroyed their distinctive characters. This secondary development consisted in a multiplication in the number as well as a great enlargement of the cells. This caused the spores to become very irregular in form, much darker, deeply constricted, and very much larger than normal (Plate XIX: 2, 6, 10).

As a result of the secondary development, the spores of Alternaria assumed the form of Mystrosporium, as was pointed out by Constantin (4). Corda (6) in his description of the genus Mystrosporium made figures which are undoubtedly of Alternaria spores in an advanced stage of secondary growth. Later (6) he figured the development of *M. stemphylium* Corda from the regular form of a young Alternaria spore through the various stages of secondary development to the very irregular form called Mystrosporium. From the descriptions of species of Mystrosporium there is no doubt that most if not all of them belong to Alternaria. All specimens labeled Mystrosporium which I examined in the exsiccati were Alternaria in various stages of secondary enlargement.

Young colonies of all species of Alternaria produced smooth regular spores which remained regular for a time interval which varied in different species and on different media. The richer the medium the earlier the secondary enlargement began. The first indications of this secondary growth were multiplication of and deep constriction at the septa. On leached agar no secondary enlargement occurred in any species, even after a lapse of three or four months. There occurred irregularly in all species a well-marked echinulation of the spores. Beyond a deep constriction at the septa, no secondary change was noted in Macrosporium, nor in *A. sonchi*, *A. brassicae* var. *microspora*, and *A. solani* from potato. This last, however, produced spores in numbers sufficient for observation only on leached agar where secondary enlargement did not occur in any species.

V. DISCUSSION

A general survey of the characters upon which specific descriptions of Alternaria and Macrosporium are, or may be, based is fitting in this place.

Broadly considered, the different bases for descriptions may be divided into natural morphology, cultural morphology and media changes, and host relations. In the present studies effort was made to get a fair estimate of the value of each of these by studying them under various conditions. In specific descriptions the shape, size, and color of spores are generally given. Also septation, constriction, and echinulation are more or less frequently mentioned. In many instances one of these characters may furnish the only point of differentiation of one species from another species in the description of which that character is not mentioned. The conidiophores are generally more or less definitely described, the characters commonly given being, length, width, septation, constriction, color, branching and geniculation. As in the case of the spore characters, any one or all of these characters may be neglected by one author or made the basis for specific description by another. The nature of the submerged mycelium has been made the basis for specific descriptions in some cases, although in many cases it is not mentioned. In artificial cultures where the mycelium is easily studied it is generally taken into consideration. The characters noted are thickness, length between septations, color, constriction, fasciculation and rate of growth. Aerial mycelium is not often mentioned in specific descriptions. Length, width, color, abundance, and general habit are the characters considered.

Morphology

1. Spore: The previously described experiments show that, within the natural range, the size of the spores under natural conditions was, in almost all of the species studied, quite constant, although under ordinary cultural conditions the size of the spore on artificial media is usually less than that on the natural host. However, in a relatively short time—two to six weeks—in some species there is a marked secondary development.

The most characteristic thing about the spores of these fungi is their shape which is an immediate index to the genera, and generally to the species. All obclavate, cuneate, ovate, pointed or beaked spores belong to Alternaria and under suitable conditions form chains. There

is variation in shape within a single species, but in most cases under natural conditions the shape of the spore, combined with its size, identifies the species. Parasites, as they are collected during the period of active attack upon plants, are uniform in spore form. Some species wintering on decaying leaves showed a secondary development which quite destroyed their characteristic form and size and made recognition impossible. On artificial media where secondary growth occurred frequently, the shape of the spores was greatly changed.

All Alternaria spores have a more or less acute apex and some species have beaks. In some cases the beaks are six or seven times the length of the spore itself. This, however, occurs only when they are not borne in chains, or in the terminal one of a chain. In the chains the terminal spore is formed by budding out of the apex, or occasionally from the side, so that the spore above takes the place of the beak of the spore below. For this reason the length of beak alone cannot be taken as of great value. Many species of Alternaria do not have beaked spores, so to a certain extent the beak is a specific character.

There is marked difference between species in the number of septa. Septation is dependent on the size of the spore in a single species, but independent of the size of spore in different species. The amount of septation increases with the age of the spore. The same conditions which brought about changes in shape and size of spores, increased the number of septa and constriction at the septa.

Echinulation occurred at times in all of the species studied. In some cases part of the spores from a colony were echinulate while others were smooth.

When the age of the spore is considered, color is an important character of some species. Generally spores darken with age. *Alternaria dianthi* and *A. brassicae* var. *microspora* grown at 10° showed marked color change, both being very light amber instead of the normal dark olive. *A. fasciculata*, forming spots on partially etiolated cabbage leaves and also in growing on raw disinfected cabbage leaves and petioles, showed a similar change.

2. Conidiophore: The form of the conidiophore is often quite characteristic, although length depends upon the age and environment. In *Macrosporium sarcinaeforme* the end of the conidiophore becomes swollen before the spore appears. If this spore falls off another bud forms at the end of the conidiophore, producing another "joint" which in turn swells at the end and produces a spore. If the spore

does not fall off, the conidiophore may bud again at the side of the swollen end and produce a second short "joint" which bears a second spore. This process, repeated, causes the geniculation which is sometimes given as a specific character. Branching occurred occasionally in all the species but was not often observed under natural conditions. The width of the conidiophores was quite constant. Constriction differs between species and with the age of the conidiophore. In some species no constriction was noted while in others the constriction was marked.

Color is as distinctive of the conidiophores as of the spores and is usually concolorous with that of the spores. The only variations in the color of conidiophores on artificial media corresponded with the color change of spores.

3. Aerial mycelium: Parasitic species under usual conditions produce little or no aerial mycelium. Under humid conditions, a very little aerial mycelium was sometimes produced by some species. This was always lax and white, and although in some species it was thicker in cross section than in others, it was so nearly featureless as to be thought worthless in classification. More or less aerial mycelium was produced by all species under some conditions of artificial culture. In some species, notably *A. brassicae* var. *microspora* and *M. sarcinaeforme*, it could be found only with the microscope. Other species produced aerial mycelium in abundance under humid conditions on rich media.

4. Submerged mycelium: The characters of the submerged mycelium were the most variable of all characters studied. Under ordinary cultural conditions on standard media the mycelium of some species had distinguishing characteristics, but any change in the cultural conditions might bring about a change in the characters of the mycelium which would make its identity doubtful. On low-grade nutrient agar the mycelium of most species was colorless and, with a few exceptions, characterless. On some media the color, width, and general character might separate certain species from certain other species, but under the influence of strong inhibition, such as was brought about by bacterial influence, the changes entirely destroyed any distinguishing characters. In most cases age greatly affected the mycelium in color, septation, and constriction.

Host Relation

Inoculations upon host plants show that some species are quite narrow in their range; *e. g.*, *A. sonchi* was parasitic on *Sonchus asper* and *Lactuca canadensis* but failed to maintain itself on cultivated lettuce and other Compositae. Others may be broader in their range, growing actively on many species of one family and less actively on other families; as, *A. solani* which grew actively on all solanaceous hosts used and less actively on cabbage. *A. brassicae* var. *microspora* grew actively on all cruciferous hosts inoculated but failed in all inoculations outside of that family. Forms morphologically inseparable may grow on different hosts and fail to cross-inoculate, or of several forms morphologically alike only one strain may be parasitic. On the other hand, forms which are morphologically clearly distinct may be equally parasitic on the same host.

The studies here presented would seem to show that the present tendency toward limiting the distinction of species to differences in morphology, regardless of host relationships, is commendable. They would seem to indicate, further, that characters should be sought which are least affected by cultural conditions, and in using such characters due consideration should be taken of factors liable to make those characters variable; such as, age or stages in development.

VI. GENERIC LIMITATIONS OF ALTERNARIA AND MACROSPORIUM

A. Alternaria

In every case where species of Alternaria have been described, the spores have been more or less elongated and pointed at one end. In the present studies all spores of the obclavate, cuneate, or ovate form produced chains of spores under favorable conditions. Since the species studied in culture covered all the types of spores found in exsiccati or described in the "Sylloge Fungorum" under the genera Alternaria and Macrosporium, there is no doubt that all of the species with these types of spores belong to the genus Alternaria, that most of the species named under Macrosporium belong to this genus, and can be recognized as such by the descriptions given of their spores.

The term "clavate," as applied to the Alternaria spores, is a misnomer arising from a misconception and carrying misconception with it. Without exception the pointed end of the spore is the apex and the rounded end is the base. *Alternaria tenuis* has been figured with its spores attached by their apices, and as this error was widely

copied it undoubtedly went far toward spreading the misconception. Other species of Alternaria, described as Macrosporium, have been similarly figured and it is a common conception that the beak is a pedicel. Many authors have made correct descriptions and figures Alternaria. In such cases the term "obclavate" replaces "clavate."

The generic description of Alternaria[4] should be emended as follows:

ALTERNARIA Nees. Conidiophores solitary or fasciculate, erect or subdecumbent, simple or branched, generally short, colored. Conidia muriform, often with few longitudinal septa, ovate, obclavate, or elongate, always with more or less definitely pointed apex, often long-beaked, colored; under favorable conditions forming chains. (Ex., *A. tenuis*, the type of the genus.)

B. MACROSPORIUM

Of the four species of Macrosporium described by Fries when he created the genus, *M. convallariae* and *M. cheiranthi* undoubtedly belonged to Alternaria. Fries, however, had rejected the genus Alternaria and placed *A. tenuis* in the genus Torula as *T. alternata*. In the exsiccati studied, specimens labeled as *M. convallariae* and *M. cheiranthi* belonged to Alternaria. The third species of Fries, *M. tenuissimum* (*Helminthosporium tenuissimum* Nees), is placed by Saccardo in the genus Clasterosporium as *C. tenuissimum*. The fourth species of Fries's original publication, *M. caracinum*, is not mentioned by Saccardo. The material for this species was supplied to Fries (7) by Schweinitz, and the description of Fries corresponds exactly with the description and figures of Schweinitz (18) for *Clasterosporium caricinum*, which is the type of the genus Clasterosporium. There is no doubt, therefore, that the types of *Clasterosporium caricinum* and *Macrosporium caricinum* are from the same material.

Corda (6) placed *Clasterosporium caricinum* in the genus Sporadesmium as *S. closteriosporium*, but the genus Sporodesmium was at that time so heterogeneous that such a disposition cannot be considered final.

Since *Macrosporium caricinum* Fries and *M. tenuissimum* (Nees) Fries were published two years before *Clasterosporium caricinum* Schw.,

[4] ALTERNARIA Nees, Syst. d. Pilze 11, p. 72 (Etym. *alternus* ob conidia alterne crassiora et tenuata). Hyphae fasciculatae, erectiusculae, subsimplices, breves. Conidia clavato-lageniformia septato-muriformia per isthmos (conidiorum caudas) catenulatim digesta, mox vero secedentia.—*Polydesmi*, *Macrosporii* et *Clasterosporii* species nonnullae obiter observatae forte huc spectant. (Saccardo, Sylloge Fungorum IV: p. 545.)

M. tenuissimum (Nees) Fries becomes the type of the genus Macrosporium, which will take over the species now given under the genus Clasterosporium, Clasterosporium being antedated by Macrosporium.

All but twenty of the species of Macrosporium listed in the "Sylloge Fungorum" belong to Alternaria. These twenty are given as having globular and sarcinaeform, or globular and clavate spores. The latter species may possibly have both forms of spores but it is much more probable that in most cases the "clavate" spores belong to Alternaria and that two distinct forms were present when the species were described. Such mixtures were found in the exsiccati in several instances. Of the two species studied in culture *M. sarcinula* very rarely produced a pointed spore bearing another spore and might be considered a bridging species to Alternaria.

All of the twenty species given in the "Sylloge Fungorum" having sarcinaeform or globose spores are one species in so far as spore measurements are dependable. Of the thirteen specimens found in exsiccati, however, there were two distinct forms, and two distinct forms were cultured during the present studies. Some of the names of the twenty species in question have already been reduced to synonymy and it is probable that only a few separable forms exist.

There is nothing in the morphology of the species of this group which would exclude them from the genus Stemphylium.

The species now named Macrosporium which should be put into Stemphylium, providing they are distinct species, are: *M. sarcinula* Berk, *M. sarcinaeforme* Cav., *M. abruptum* C. & E., *M. baccatum* Ell. & Kell., *M. valerianellae* Roum., *M. elegantissimum* Rabh., *M. pelargonii* E. & E., *M. globuliferum* Vestergr., *M. toruloides* E. & E., *M. nodipes* Sacc., *M. schemnitziense* Baumel., *M. puccinioides* E. & And., *M. chartarum* Pk., *M. myrmecophilum* (Fr.) Sacc., *M. nitens* (Fr.) Sacc., *M. subglosum* Cke. & Rav., *M. rosarium* Penz., *M. septosporum* Rabh., *M. atrichum* C. & E., *M. parasiticum* Thum., *M. commune* Rabh.

VII. SPECIFIC LIMITATIONS OF ALTERNARIA

Except for the species named above as belonging to Stemphylium, all the species of Macrosporium in the "Sylloge Fungorum" belong to Alternaria, and can be recognized as Alternaria from their descriptions.

In the present studies enough variation due to age alone occurred in *Alternaria tenuis*, *A. fasciculata*, and *A. dianthi*, to fit these to a

large percentage of the species of Alternaria and Macrosporium described in the "Sylloge Fungorum" and found in the exsiccati. No variations occurred in the forms in group *6*, of the exsiccati material studied except those which would occur due to age, and all in this group might be considered *A. tenuis* and varieties of *A. tenuis*. Even the narrowest limitation in the application of specific names would preclude the retention of many of the present species.

No final disposition of the present specific names of Alternaria and Macrosporium can be made without a study of authentic specimens of each species. Most of the descriptions are far from being complete or definite enought to permit their being used for this purpose. For convenience a tentative grouping of similar forms which may be identical, and which are undoubtedly closely allied, should be made. These groups might well be retained to indicate the similarity of a number of forms such as is exemplified in bacteriology in the *B. coli* and *B. subtilis* groups. As in bacteriology, each group should be designated by a typical species.

The groups suggested are as follows:

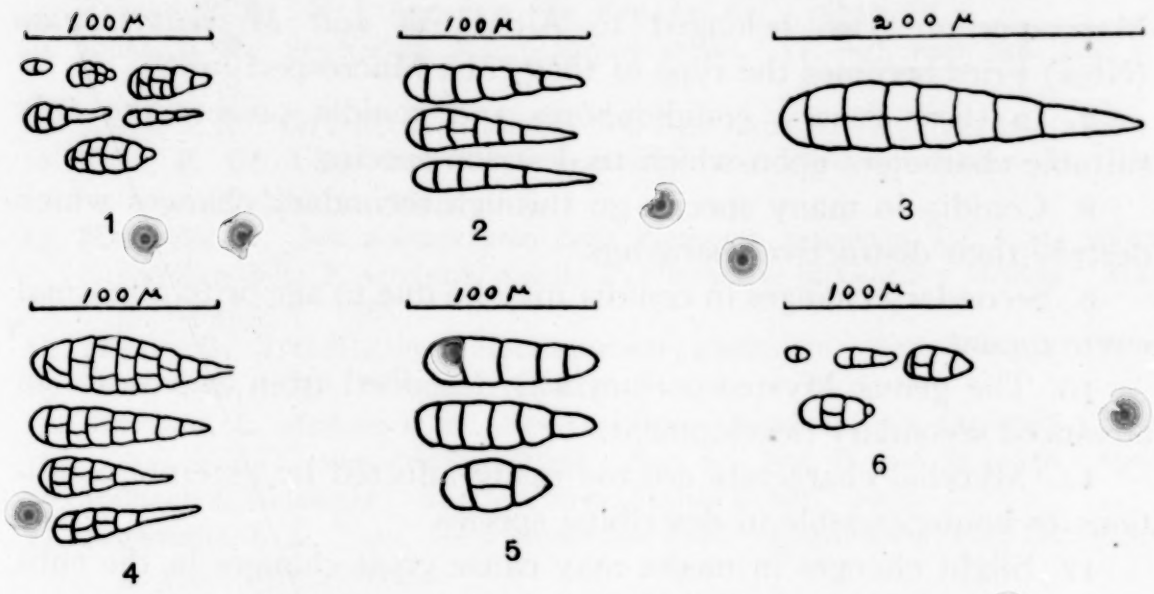
The *A. tenuis* group. This group is characterized by spores ranging from 11–50 × 7–20 μ. The spores are quite variable in form as well as in size but are generally broad and muriform (fig. 1, spores of the *A. tenuis* group). All specimens mentioned in group *6*, page 446, belong here.

The *A. brassicae* group. This group should contain regular, long, tapering, acute-beaked spore forms with measurements ranging from 35–120 × 10–30 μ. The spores have few longitudinal septa and are often long beaked (fig. 2, spores of the *A. brassicae* group). All of group *3*, page 444, belong in this group.

The *A. herculea* group. *A. herculea* (E. & M.) com. nov. (*Macrosporium herculeum* E. & M.) is the type of a group with spores similar in form to those in the *A. brassicae* group but much larger (fig. 3, spores of the *A. herculea* group). To this belong the specimens mentioned in group *4*, page 445.

The *A. cucumerina* group. *A. cucumerina* (E. & E.) com. nov. (*Macrosporium cucumerinum* E. &. E.), syn. *A. brassicae* var. *nigrescens* Peglion, is typical of a group similar in spore form to that of *A. brassicae* but with the spores uniformly wider and more muriform and, generally, shorter (fig. 4, spores of the *A. cucumerina* group). In this group should be placed all in group *5*, page 445.

The *A. sonchi* group. *A. sonchi* Davis is different from any of the species in the above groups in having large-celled spores with a markedly obtuse apex (fig. 5, spores of the *A. sonchi* group). The range in size is 50–125 × 12–25 μ. No similar form was found in exsiccati.



TEXT-FIGS. 1–6.—For explanation see text.

The *A. brassicae* var. *microspora* group. The spores of *A. brassicae* var. *microspora* (Berk.) Sacc. differ from those of *A. tenuis* in being uniformly narrower and less muriform; longitudinal septa seldom being formed (fig. 6, spores of the *A. brassicae* var. *microspora* group). Here belong those of group 7, page 447.

A more complete study of exsiccati might show the necessity of other groups for forms not included above.

Without doubt the number of valid species of Alternaria is a very small percentage of the present named species of Alternaria and Macrosporium, but it is outside of the limits of the present paper to reduce these names to synonymy.

VIII. SUMMARY.

1. Spore shape in the genus Alternaria is correlated with catenulation of spores.

2. All obclavate, ovate, cuneate, or elongate-pointed spores of the Macrosporium-Alternaria type form chains and belong to Alternaria.

3. The acute end of the spore is the apex or beak, not the base or pedicel.

4. Of these spores, all globular, sarcinaeform, cubed, or oblong spores without apex or beak belong to Stemphylium.

5. Macrosporium, by priority, becomes the name of the genus which has been known as Clasterosporium Schw.

6. Of the four species described by Fries when he created the genus Macrosporium, two belonged to Alternaria and *M. tenuissimum* (Nees) Fries becomes the type of the genus Macrosporium.

7. In these genera, conidiophores and conidia possess the only suitable characters upon which to describe species.

8. Conidia in many species go through secondary changes which destroy their distinctive characters.

9. Secondary changes in conidia may be due to age or to abnormal environment.

10. The genus Mystrosporium was described from Alternaria in advanced secondary development.

11. Mycelial characters are too easily affected by external conditions to be dependable in describing species.

12. Slight changes in media may cause great changes in the submerged mycelium.

13. Identical changes of environment may bring about opposite reactions in different species.

14. Morphology of the dependable stable characters under normal conditions is the most useful basis for describing species.

15. Due consideration must always be taken of the age of the organism in describing a species.

16. For convenience, the genus Alternaria should be divided into groups of species having similar spores.

The writer takes this opportunity to express his thanks to Professor F. L. Stevens for encouragement and many helpful suggestions in carrying out this work; to Professor Wm. Trelease for aid in solving the taxonomic problems involved; and to others, mentioned or not mentioned in this paper, who have given assistance in any way.

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EXPLANATION OF PLATES XIX AND XX

Plate XIX

All drawings were made with camera lucida to scale shown on the plates.

1. *Alternaria brassicae* var. *nigrescens* on leached agar.

2. *Alternaria brassicae* var. *nigrescens* spores showing stages in secondary development.

3. *A. solani* from Datura on leached sugar.

4. *A. solani* from Datura, spores from bean agar culture.

5. *A. solani* from potato on leached agar.

6. *A tenuis* spores two weeks and six weeks old showing secondary development due to age.

7. *A. tenuis* on leached agar.

8. *A. dianthi* on leached agar.

9. *A. dianthi* spores from colony on acid agar.

10. *A. dianthi* spores from colony on bean agar at 10°, showing enlarged size and secondary development.

11. *A. fasciculata* on leached agar.

12. *A. fasciculata* spores showing echinulation.

13. *A. fasciculata* showing development of a spore.

14. *A. brassicae* var. *microspora* on leached agar.

15. *A. brassicae* var. *microspora* on bean agar, 10°, showing reduced size of spores and lack of color.

16. *Macrosporium sarcinaeforme* on leached agar.

17. *M. sarcinula* on leached agar.

PLATE XX

1. Mycelium of *A. solani* from potato; *a*, reaction to bacterial influence; *b*, normal.

2. Mycelium of *A. brassicae* var. *microspora; a* and *b* as in No. 1.

3. Mycelium of *A. brassicae* var. *nigrescens; a* and *b* as in No. 1.

4. Mycelium of *A. solani* from Datura; *a* and *b* as in No. 1.

5. Mycelium of *A. tenuis; a* and *b* as in No. 1.

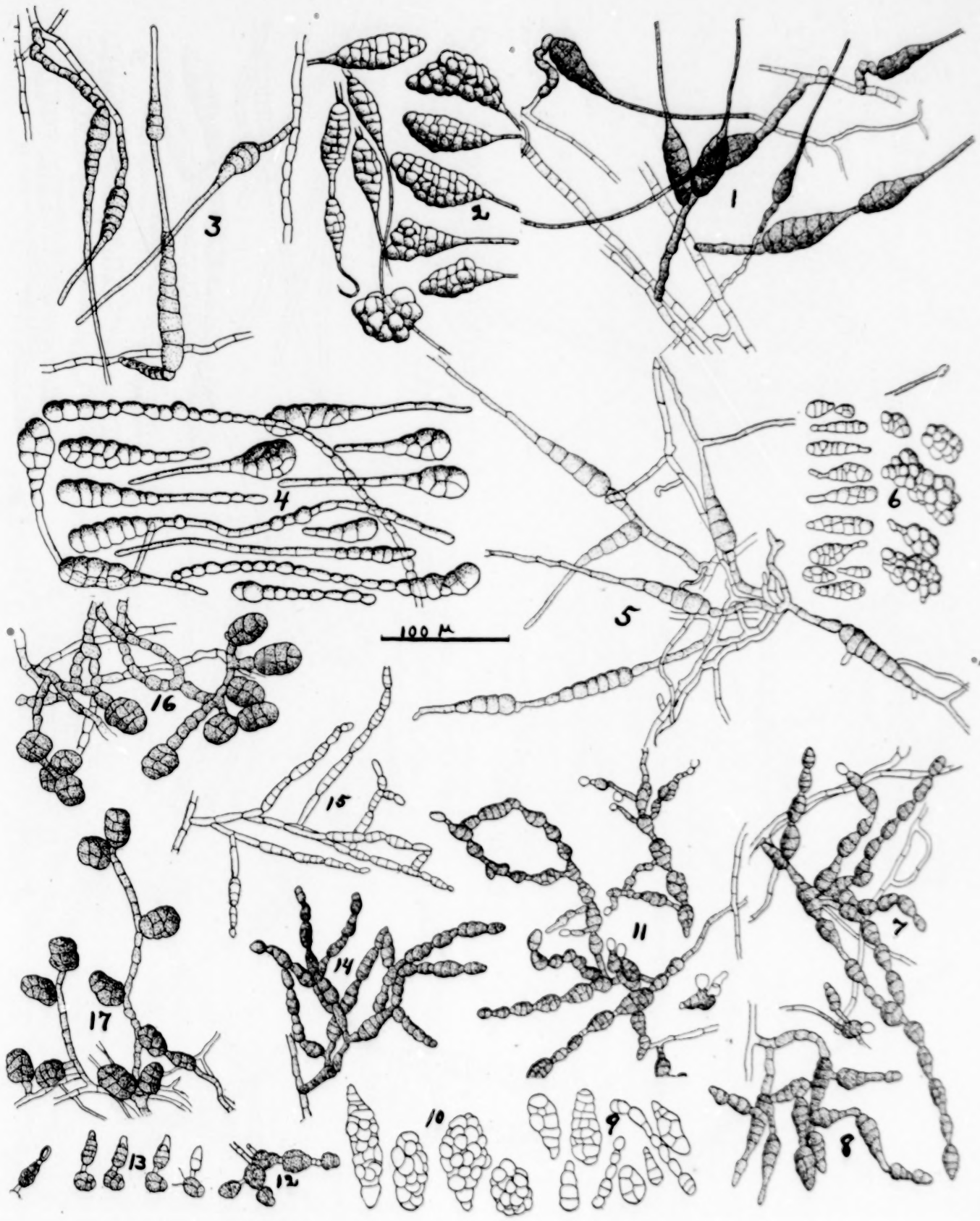
6. Colony of *A. brassicae* var. *microspora* showing reaction to Colony of *B. x.*

7. *A. iridicola* on leached agar.

8. *A. sonchi* conidiophores and spores on *Lactuca canadensia.*

9. *A. sonchi* conidiophores and spores on leached agar.

10. Spores of *A. solani* from Datura; *A*, from Datura leaf spot; *B*, from potato leaf spot; *C*, from tomato leaf spot (artificial inoculations).



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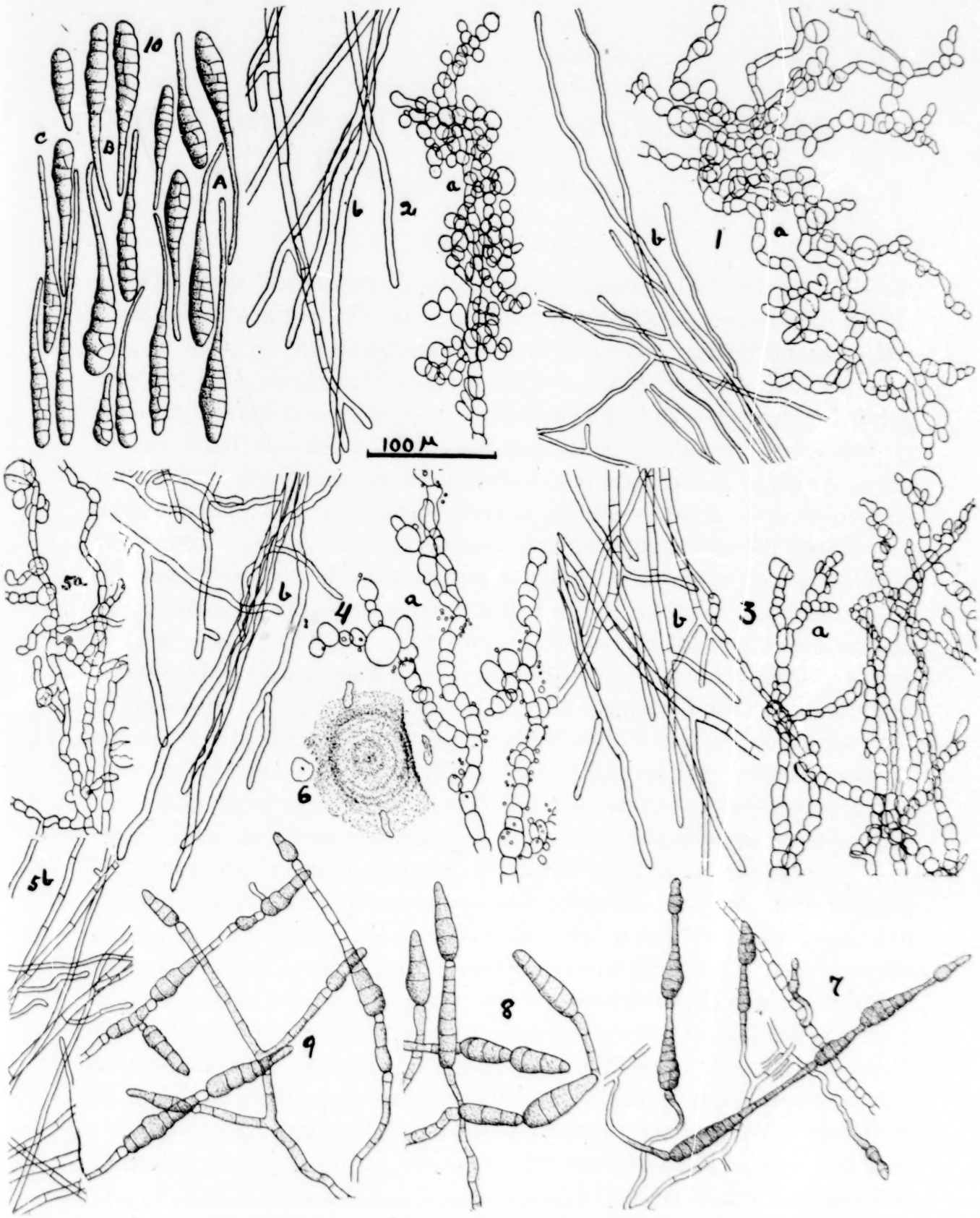

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